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• ESOPHAGEAL CANCER •

HLA-DRB1 allele polymorphisms in genetic susceptibility to esophageal carcinoma

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Abstract

AIM: To probe into the genetic susceptibility of HLA-DRB1 alleles to esophageal carcinoma in Han Chinese in Hubei Province.

METHODS: HLA-DRB1 allele polymorphisms were typed by polymerase chain reaction with sequence-specific primers (PCR-SSP) in 42 unrelated patients with esophageal cancer and 136 unrelated normal control subjects and the associated HLA-DRB1 allele was measured by nucleotide sequence analysis with PCR.SAS software was used in statistics.

RESULTS: Allele frequency (AF) of HLA-DRB1*0901 was significantly higher in esophageal carcinoma patients than that in the normal controls (0.2500 *vs* 0.1397, $P=0.028$, the odds ratio 2.053, etiologic fraction 0.1282). After analyzed the allele nucleotide sequence of HLA-DRB1*0901 which approachs to the corresponded exon 2 sequence of the allele in genebank. There was no association between patients and controls in the rested HLA-DRB1 alleles.

CONCLUSION: HLA-DRB1*0901 allele is more common in the patients with esophageal carcinoma than in the healthy controls, which is positively associated with the patients of Hubei Han Chinese. Individuals carrying HLA-DRB1*0901 may be susceptible to esophageal carcinoma.

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INTRODUCTION

The major histocompatibility complex (MHC) refers to as human leukocyte antigen (HLA). The loss of HLA antigens by neoplastic cells is considered important for tumor growth and metastasis[1-3]. Since tumor neoantigens on the surface of aberrant cells are recognized by T-cells only in the context of the HLA "self" antigens, loss of the HLA antigens may allow the tumors to escape immunosurveillance[4]. HLA system is a kind of genetic marker of human being, and the most complicated human genetic polymorphic system with hereditary features of haplotype inheritance and allele polymorphism and linkage disequilibrium. It played an important role in the event of antigen recongnition and presentation, immune response and modulation, destroying foreign antigen targeted cells. The alleles of the HLA system control a variety of immune functions and influence the susceptibility to more than 40 diseases, many of which have an autoimmune component[5-17], esophageal cancer is a complex, probably multifactorial disease[18-41]. Association of a particular HLA allele with a disease implies that the frequency of the allele is different in the patient population as compared with that of an ethnically matched control population. However, there has been no report on the association between HLA alleles and esophageal carcinoma.

In this study, we used polymerase chain reaction with sequence-specific primers (PCR-SSP) and DNA sequence analysis techniques on HLA-DRB1 alleles typing to investigate the genetic susceptibility of HLA allele polymorphisms in esophageal carcinoma of Hubei Han Chinese. This may be beneficial to the early prevention and surveillance,thus setting up gene therapy basis for esophageal carcinoma.

MATERIALS AND METHODS

Subjects

Included in our study were healthy controls and patients with esophageal carcinoma. The control group consists of one hundred and thirty-six unrelated donors or healthy individuals by physical examination, including 62 men and 74 women, ranging 22-48 years, in age, with a mean of 36±6 years. The esophageal carcinoma group includes forty-two unrelated patients with esophageal squamous cell carcinoma, 35 men and 7 women, ranging in age 41-80 years,with a mean age of 60±5 years,who were evaluated endoscopically and surgically. And all were tested by histopathology at Zhongnan Hospital of Wuhan University, between August 1998 and June 1999.

DNA extraction

Genomic DNA was isolated from leukocytes obtained from anticoagulated peripheral blood of patients and controls using the salting-out procedure[5,42,43], or QIAamp Blood Kit (QIAGEM GmbH, Germany) with which DNA was obtained through solid phase affinity columns.

HLA-DRB1 alleles PCR-SSP typing

For HLA-DRB1 "low solution" typing by PCR-SSP, 23 separate PCR reactions were performed for each sample. PCR-SSP typed system: each PCR reaction mixture contained 2-4 allele- or group-specific -DRB1 primers and the internal positive control primer pair. Allele sequence specific primers (2pmol), designed on the basis of published sequences[43-45], were used in multiple amplification reaction. HLA-DRB1 alleles PCR-SSP typed system consisted of 60 ng genomic DNA, 0.5 U *Taq* DNA polymerase (Ampli Taq® DNA polymerase, Roche Diagnostic System, Inc. USA), 20 μmol

each deoxyadenosine triphosphate (dATP), deoxycytidine triphosphate (dCTP), deoxyguanosine triphosphate (dGTP), deoxythymidine triphosphate (dTTP), 10 mmol/L Tris-HCl pH8.3, 50 mmol· L^{-1} KCl (kalium chloride), 1.5 mmol· L^{-1} $MgCl_2$ (magnesium chloride). PCR amplifications were carried out in PTC-100 thermal cycler (MJ Research, Inc, USA) according to the method of Olerup *et al*[5,42,43]. Initial denaturation was made at 94 ℃ for 5 min; with 30 cycles each consisting of denaturation at 94 ℃ for 30 s, annealing at 65 ℃ for 1 min and extension at 72 ℃ for 1 min. The HLA-DRB1 alleles typed vizualization of amplification was observed using medium resolution PCR-SSP products by 20 g· L^{-1} gels agarose(Boehringer Mannhein GmbH, Germany) electrophoresis. The gels were run for 20 min at 15 V· cm^{-1} in 0.5xTBE buffer and visualized using UV illumination and keeping file copies in computer.

Positive control, false negative allele

The most common form of individual PCR reaction failure is where random individual reactions fail to produce allele or control bands. This occurred on average in 1 % of all PCR-SSP amplification. In each PCR reaction, a pair primers were included which specifically amplify the exon 2 of HLA-DRB1 alleles. These two primers matched non-allelic sequences and thus functioned as an internal positive amplification control. We used human growth hormone gene as a intra-positive control, in which primer[5] is 5' -primer, 21 mer, 5' GCC TTC CCA ACC ATT CCC TTA 3' , Tm64 ℃; 3' -primer, 22 mer, 5' TCA CGG ATT TCT GTT GTG TTT C 3' ,terminal concentration 0.15 μmol· L^{-1},product 429 base pair (bp) fragment. Control failure is not a problem if the genotype obtained is heterozygous for all alleles and the type is unequivocal. Homozygous samples, in which the control failed, normally would require typing with a new DNA sample once again. Individual false negative allele amplifications where the control amplification worked but an expected allele was not amplified, did occur, the same be required repeated typing.

DNA sequence analysis of PCR-SSP products

Specific PCR-SSP products of amplification were obtained from agarose gels electrophoresis, then purified with glassmilk kit (Clontech Laboratories, Inc, USA), and the base sequence was examined by PCR sequence analysis with ABI prism 310 (Perkin-Elmer, USA) with the addition of a terminal deoxytransferase extension step at the end of the chain termination reaction.

Statistical analysis

SAS (6.12 for Win), including χ^2 analysis or Fisher' s Exact Test,was used to compare the allele frequency (AF) of HLA-DRB1 between the patients with esophageal carcinoma and the controls.

RESULTS

HLA-DRB1*0901 was present at increased frequency in patients with esophageal squamous cell carcinoma, 0.2500 *vs* 0.1397, *P*=0.028, odds ratio 2.053, etiologic fraction 0.1282 (Table 1). The rested HLA-DRB1 alleles frequencies showed no significant difference in comparison between patients and the controls, i.e., there was positive association between HLA-DRB1*0901 and the patients of Hubei Hans. The HLA-DRB1*0901 nucleotide sequence, was analyzed in this study, approachs to the corresponded exon 2 of the allele sequence in genebank. Esophageal carcinoma was associated with HLA genotype: individuals carrying HLA-DRB1*0901 may be susceptibilitive to esophageal carcinoma in Hubei Hans.

Table 1 HLA-DRB1 allele frequencies in esophageal cancer patients of Hubei Han Chinese

HLA-DRB1 allels	Control group			Esophageal cancer group			*P*
	N1	AF(n_1=272)	PF(n_2=136)%	N2	AF(n_1=84)	PF(n_2=42)%	
0101-2	13	0.0478	9.5588	2	0.0238	4.7619	>0.05
0103	0	0.0000	0.0000	0	0.0000	0.0000	
150X	46	0.1691	32.3529	9	0.1071	21.4286	>0.05
160X	9	0.0331	6.6176	3	0.0356	7.1429	>0.05
0301	19	0.0699	13.9706	6	0.0714	14.2857	>0.05
0302	2	0.0074	1.4706	0	0.0000	0.0000	>0.05
040X	30	0.1103	20.5882	12	0.1429	26.1905	>0.05
0701-2	13	0.0478	9.5588	3	0.0357	7.1429	>0.05
080X	22	0.0809	15.4412	4	0.0476	9.5238	>0.05
0901	38	0.1397	26.4706	21	0.2500	45.2400	0.028*
1001	11	0.0404	7.3529	2	0.0238	4.7619	>0.05
110X	18	0.0662	12.5000	7	0.0833	16.6667	>0.05
120X	17	0.0625	12.5000	11	0.1310	26.1905	>0.05
1301-2	15	0.0551	11.0294	1	0.0119	2.3810	>0.05
1303-4	4	0.0147	2.9412	0	0.0000	0.0000	>0.05
1305	1	0.0037	0.7353	0	0.0000	0.0000	>0.05
1305-6	0	0.0000	0.0000	0	0.0000	0.0000	
140X	15	0.0551	11.0294	3	0.0357	0.0357	>0.05

AF: allele frequence, PF: phenotype frequence;
P: Fisher's exact test (2-tail) or χ^2, compared with the control with AF;
*Odds ratio=2.053, etiologic fraction=0.12820.

DISCUSSION

Familial aggregation of esophageal cancer is common. There is an approximate increase in abnormal chromosome ratio of this cancerous relatives as compared with the general population,although the inheritance patterns clearly fit no simple Mendelian patterns. However, the illness may exist in the same family at a higher frequency than expected by chance alone[5,24-27]. This suggests that there may be an internal environment susceptible to malignant and a genetic component in the patients' families,which supports the concept that heredity may play an important role in the pathogenesis of esophageal cancer[2, 9, 46-53].

Major histocompatibility complex (MHC) is a genetic name describing alleles encoding antigens first discovered because they determine in a major way the fate of a graft, i.e., histocompatibility. In many species, the MHC has an additional name such as HLA for humans, H-2 for mice, SLA for swine, etc. The HLA alleles are located in a 3 500-4 000 kilobase region of chromosome 6; and the allele encoding β2-microglobulin, a related protein in the system, is on chromosome 15. The major classes of HLA alleles are class I (HLA-A, -B, and -C) and class II (HLA-DR, -DQ, and -DP).Between the class I and II alleles, there are many other alleles, some with immune-related functions that could also be associated with diseases,tumor necrosis factor A and B genes being among them. Class II HLA presents peptides derived from extracellular antigens. The HLA polymorphism appears to be responsible for variations in the immune response of different individuals to different antigens, and may contribute to the susceptibility to diseases and autoimmune disorders[5,15-17]. The loss of HLA antigens by neoplastic cells is considered important to tumor growth and metastasis, and for tumor escape immune surveillance. HLA class I molecules are required for the presentation of tumor neoantigens to cytotoxic T-lymphocytes. There is evidence that tumor cells with reduced expression or lack of such antigens could evade an immune response and selected for tumor progression. It can be considered that either extensive abnormalities in the regulation of the HLA alleles occurred or substantial chromosomal damage took place in the short arm of chromosome 6, where the human HLA allele complex is located. It was demonstrated that oncogenes may suppress the expression of HLA class I alleles, such as the activation of oncogenes or the inactivation of suppressor-genes[50,54-56]. The data presented here demonstrate that HLA-DRB1*0901 AF increased in the patients with esophageal cancer compared with that in healthy controls (0.2500 *vs* 0.1397, *P*=0.028, OR=2.053, EF=0.1282), but none of the rested HLA-DRB1 alleles occurred at markedly altered frequency between the patients and the normal individuals we investigated, indicating that HLA-DRB1*0901 is positively associated with esophageal cancer.

The nucleotide sequence of HLA-DRB1*0901 allele which was measured in our research approachs to the corrsponded exon 2 gene sequence of genebank[44,45]. The AF of HLA-DRB1*0901 was also increased in both Japanese patients with lung cancer and prostate cancer. It is the allele that is associated with genetic susceptibility of various tumors,but why? It was entirely unclear up to now. Pathogenesis of genetic association may be linkage disequilibrium (nonrandom association) and/or changing in the recognized procession of the specific antigen.

It is still controversial whether or not HLA antigen expression in carcinomas correlates with the development of carcinoma and prognosis. The immune responses involving HLA antigens expressed on carcinoma cells are thought to play an important role in eliminating mutated cells or suppressing carcinoma progression[51-53,57-59]. As reported in some studies, the reduced expression of HLA antigens in malignant tissues has been proposed as a mechanism thereby tumor-associated proteins cannot be presented in the T cells, therefore the tumor cell proliferates are unperturbed by the immune system and carcinomas protect themselves from hosts' immunosurveillance. There is a possibility that HLA allele genetic association and expression on carcinoma may provide a clue to the understanding of the therapeutic mechanisms of biological response modifiers or immunotherapy which may cut through the induction of HLA antigens on carcinoma cells[56,60-63]. The cells of a given individual may express HLA alleles, which altered binding to tumor peptides, thereby leading to a modified immune response to the tumor. Identification of the mechanism associating HLA-DRB1*0901 with esophageal cancer could ultimately help target individuals most likely to benefit from cancer screening and prevention programs, and could facilitate novel therapeutic strategies for cancer immunoprevention .

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